Recent Research in Science and Technology 2012, 4(2): 24-26
ISSN: 2076-5061
Available Online: http://recent-science.com/

Pathogenic effects of nematode parasite *Eustrongylides* sp. larvae on serum LH level and histology of gonads of freshwater fish, *Clarias gariepinus*

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Abstract

Present study was conducted to known the pathological effects caused by nematode parasite, *Eustrongylides* sp. larvae on serum LH level and histology of gonads of freshwater fish, *Clarias gariepinus.* The nematode parasite *Eustrongylides* occurs in the body cavity of its host *Clarias gariepinus* and inhibits gonadal development. A significant decrease in the gonadotropin hormone (LH) in the blood stream was recorded. LH reduction was recorded to be the 50% less as compared to the normal fishes. Histopathological studies of testes revealed disorganized testicular structure, germinal epithilium was disrupted and reduction in spermatozoa was reported. Histology of infected ovaries showed the appearance of interfollicular spaces, degeneration of follicular wall tissues and their egg envelops ruptured observed.

Keywords: *Eustrongylides, Clarias gariepinus*, Gonadotropin hormone (LH)

INTRODUCTION

Fishes contribute a lot to country's economy especially in India where there is an abundance of freshwater reservoirs of perennial rivers. Fish is one of the best sources of protein, being low in cholesterol and fat.Therefore more aquaculturists are being involved in aquacultural activities to meet out the daily requirements. Furthermore, the aquaculture has gone to a level of semi-intensive and intensive which has lead to the problem of overcrowding, poor environmental conditions in the culture system. This often results in reduced immunity of fish and higher susceptibility to parasites and diseases [1].

Parasites of fish constitute one of the major problems confronting the modern fish culturist and pathological conditions arising from parasites infection assume a high magnitude especially under crowded conditions [2]. Parasites can affect various fish organs either directly or indirectly, depending on the target organ. Generally the parasites negatively affect the reproductive effort with the decrease of its fecundity being proportional to the intensity of infection. The parasite extracts energy and nutrients from the hosts, which are not destined to reproductive effort and the parasite induces physiological, immunological or ethological changes in the host and which also impair mating, gonad maturation or larva survival [3].

Inspite of the voracious carnivorous feeding habit of *Clarias gariepinus,* it has been considered to hold great promise in fish farming in India, the fish having a wide geographical spread, a high growth rate, resistant to handling stress and well appreciated [4]. But such studies are not conducted in this fish in Indian environmental conditions. Therefore, the present study was taken up to investigate the pathogenic effects of Nematode parasite *Eustrongylides* on the reproductive function of *Clarias gariepinus*.

Received: Nov 13, 2011; Revised: Jan 04, 2012; Accepted: Feb 10, 2012.
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MATERIAL AND METHODS

Collection of fish specimens and parasites

Specimens of *Clarias gariepinus* were collected from the local fish market and also from the local fish sellers of Bhopal, India,. The fishes were brought live in the laboratory in polythene bags. Fishes were collected in plastic containers filled with water anaesthesized with the help of chloroform. Fish specimens were dissected out in physiological saline (0.75% NaCl solution) for collecting helminth parasites. Nematodes collected were washed thoroughly in normal saline. Then killed and fixed in hot 70% alcohol, stored in glycerine alcohol (1:3) and studied as wet mounts or temporary mounts in glycerine.

Measurement of serum LH level by ELISA

The blood was collected from *Clarias gariepinus* by making an incision at the caudal end, using 50ml hypodermic syringe with 26G needle. Blood samples were allowed to clot and further serum was separated by centrifugation for 20 minutes at 3000 rpm at room temperature. The enzyme immuno assay was carried out for the hormone analysis (IBL Protocol) on the basis of sandwich method. The standards in the assay have been calibrated against WHO standard (2^{nd} IRP80/558).

Histological studies

For histological studies the infected and normal gonads were carefully removed and weighed and washed out in saline water to remove blood and fixed in aqueous Bouin's fixative for 24 hours. The tissues were taken out from the Bouin's fluid after 24 hours and were washed with distilled water. They were dehydrated through graded series of ethanol, embedded in paraffin wax (melting point 58°C to 68°C). Blocks were prepared sectioned at a thickness of 5µm. The wax ribbons carrying the sections were stretched on clean glass

slides having a thin layer of albumin and kept for 24-48 hours for drying. Staining was done by the routine techniques using Haematoxylin (Delafields) and eosin which were further examined under light microscope after mounting in DPX.

Statistical analysis

Ecological analysis of parasites was done according to the method of Margolis *et al.* (1982).

RESULTS

Prevalence of parasite

A total number of 137 fishes were examined from July, 2009 to December, 2009 i.e. during spawning and post-spawning season. Out of which 26 fishes were found to be infected by *Eustrongylides* sp. larvae. Prevalence of parasite was given in figure 1.

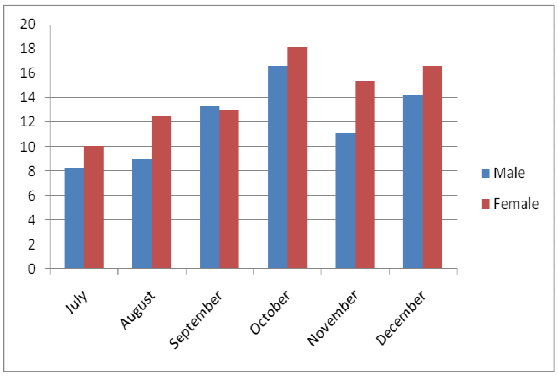

Fig 1. Showing the prevalence of *Eustrongylides* sp. larvae in infected male and female specimens of *Clarias gariepinus*

Detection of serum LH level by ELISA

The infected fishes showed a significant decrease of serum LH level when compared with the normal fishes. The serum LH level of infected fishes reduces almost to half to that of normal. The average LH level of normal female fishes was recorded to be 6.14 mIu/ml while in infected female fishes it was recorded 3.028 mIu/ml. The average LH levels of normal and infected male fishes were recorded to be 2.79 mIu/ml and 1.26 mIu/ml, respectively (Figs. 2, 3).

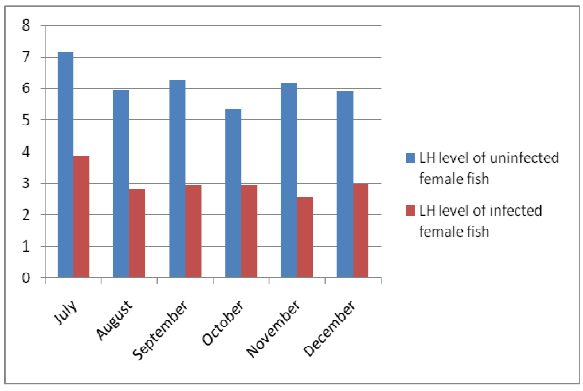

Fig 2. Showing LH level in infected and uninfected female specimens of *Clarias gariepinus*

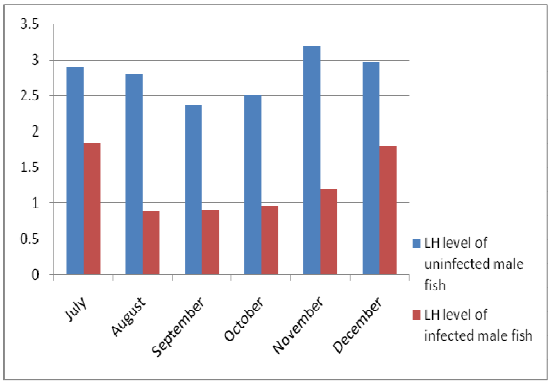

Fig 3. Showing LH level in infected and uninfected in infected male specimens of *Clarias gariepinus*

Histological studies on gonads

Histopathological studies on the testes of uninfected fishes exhibited a normal histological structure. The parasitic infection in the fish leads to an extensive damage of the testis. The primary spermatocyte walls break and separate them from seminiferous tubules. The testes showed further connective tissue damage and seminiferous tubules remain indistinguishable. Loosening of the seminiferous lobules and their lumen were also observed. The change in the seminiferous tubules was very irregular. Morphologically, the testes appeared enlarged and histologically, with more loosening and disruptive structures (Figs. 4, 5)

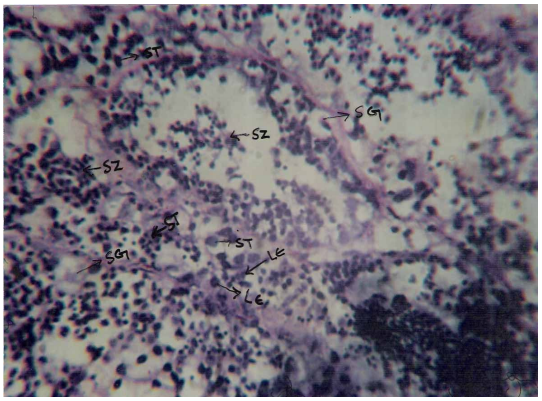

Fig 4. Microphotograph of cross section of testes showing SZ- Spermatozoa, SG- Spermatogonia, ST- Seminiferous Tubules and LE- Leydig Cells X100

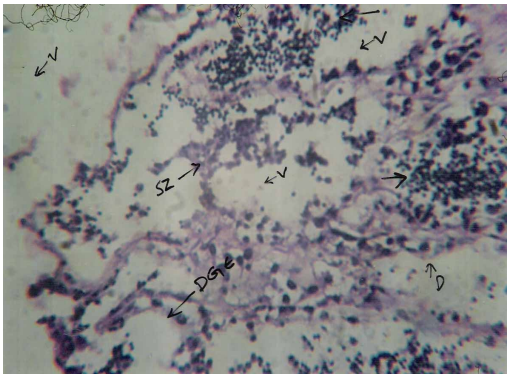

Fig 5. Microphotograph of cross section of testes showing V- necrosis, SZ- Spermatozoa, DGE- Degeneration of seminiferous tubules and D- diruptive seminiferous tubules X400

Ovaries of uninfected fishes showed normal histological structure with a number of oocytes showing various stages of maturation. Histopathological studies of infected ovary showed ovary wall become thin and slightly ruptured oocytes, immature and degeneration of egg envelope were observed and oocytes also show shrinkage, atertic follicles appear, nuclei scattered and early and late yolk stage. Ovary showed drastic reduction in size with thin wall, oocytes get detached to their envelope. Infected ovaries were also characterised by increased amount of necrosis and decrease in the formation of atretic follicles during reproductive period (Figs. 6, 7).

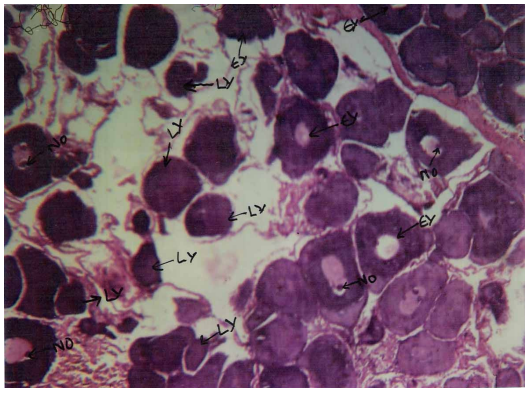

Fig 6. Microphotograph of cross section of ovary showing LY- Late Yolk Stage and RE- Ruptured Ovarian Follicle X100

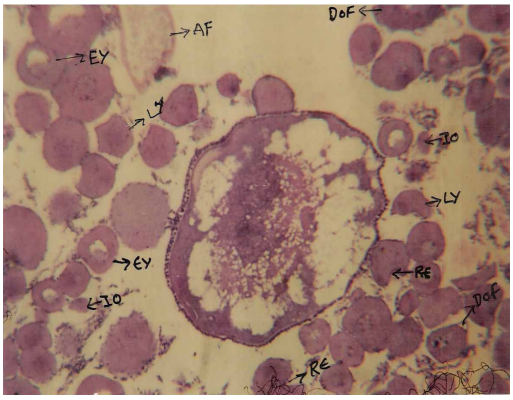

Fig 7. Microphotograph of cross section of ovary showing EY- Early Yolk Stage, EY- Early Yolk Stage, AF- Atretic Follicle, NO- Nucleolus, DOF- Disruptive Ovarian Follicle and LY- Late Yolk Stage X400

DISCUSSION

In the present investigation a profound decrease in LH was observed in the infected fishes. Similar results were reported in roach infected by *Ligula* possesed a more intricate effect on the endocrine status of a pituitary gland [5]. They also concluded that the parasites act on brain-pituitary-gonadal axis due to which hormone secretion is reduced. During present study, immature ovaries and testes were still present which may be due to the parasite is presumed to act upon the brain-pituitary-gonadal axis of the fish to inhibit further development of reproductive organs and reduction in serum LH level.

Histopathological studies of infected ovary and testes showed several alterations in normal histology of these tissues. Studies on the effect of helminth parasites on fish gonads are meager. The present finding is in agreement with those of [6], [7]. [8], [9], [10] and [11]. The present observation attributes towards that genus *Eustrongylides* sp. larvae resulted in decrease in serum LH level and further decline in the reproductive potential of infected host fish.

ACKNOWLEDGMENT

First author is thankful to Department of Zoology and Applied Aquaculture, Bhopal (M.P.), for providing infrastructure facilities.

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